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## SYNOPSIS

### EXPLORING THE IMPACT OF CHILDHOOD HBV VACCINATION IN ETHIOPIA: A SYNOPSIS AND DISCUSSION OF PRELIMINARY FINDINGS.

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## INTRODUCTION

In this issue we report preliminary results of a large multisite study intending to assess the burden of hepatitis, particularly hepatitis B virus (HBV) among children, as well as the impact of the hepatitis B vaccination program commenced several years ago (1-8). Here, we summarize key findings of this study, and discuss these results in the context of national and global issues of hepatitis B disease and control.

Hepatitis B and C infections are common in Ethiopia, with chronic disease prevalence of approximately 6-8%, and 2-4%, respectively (9). Complications of chronic hepatitis include cirrhosis with or without hepatic failure and hepatocellular carcinoma. Based on global studies, the 5-year rates of progression from chronic hepatitis B infection to cirrhosis is about 15%, from cirrhosis to hepatic failure about 20%, and from cirrhosis to hepatocellular carcinoma about 10% (10).

These complications have had limited treatment options in resource limited countries. Depending on the level of liver damage, treatment is now available for both Hepatitis B and C, but requires long term, if not lifelong therapy for chronic hepatitis B, and shorter term but extremely expensive therapy for hepatitis C (11,12). While efforts to develop a vaccine for hepatitis C are under intensive investigation, no candidates are currently available (13). In contrast, a highly efficacious vaccine for hepatitis B has been available for many years, and most countries worldwide have implemented programs to roll out universal vaccination for newborns. WHO has adopted a plan to reduce new HBV cases by 90% and mortality by 65% by 2030 (14). Since great emphasis is being placed on infant Hepatitis B vaccination as the principal control intervention for hepatitis in developing countries, it is essential that vaccination programs evaluate the effectiveness of their programs.

Most vaccination programs in Western and Asian countries have observed vaccine efficacies of 95% or greater, and a concordant drop in chronic hepatitis B prevalence over the past 20 years (15-17). Studies in sub-Saharan Africa have primarily assessed serological efficacy, that is the generation and maintenance of anti-vaccine (HBsAg) antibodies as a surrogate for clinical protection (15,18-22), but few studies have been done to assess clinical efficacy of the vaccine to prevent chronic disease, as defined by HBsAg positivity (18,21,22).

In Ethiopia, there is limited data on hepatitis infection and disease among children. A notable study was a comprehensive study in Addis Ababa performed in 1994 (23), which evaluated hepatitis B infection and disease in a large number of subjects across multiple age groups. Importantly, this study showed that chronic disease (positivity for HBsAg) was already present in 2-4% of children below 5 years of age, and that chronic disease increased to a level of 10% gradually over the next 40 years, though as many as 50% of the population could be shown to have a history of infection by serology as defined by the presence of anti-HBc and/or anti-HBsAg and/or HBsAg (23).

Given the importance of infant HBV vaccination in the strategy to control infection, and in light of the previous studies in Ethiopia, we decided to undertake a comprehensive evaluation of both the burden of hepatitis B among children and their mothers, and the efficacy of the HBV vaccine in Ethiopia. The study involved 4 sites (Addis Ababa, Gondar, Jimma and Harrar) and has been coordinated by the Armauer Hansen Research Institute.

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### **Key Findings**

Our results to date can be summarized as follows:

1. Among nearly 1500 vaccinated children from 4 sites, we observed that the overall percentage of children with protective levels of anti-HBsAg was about 60%, with a range from 32.1 to 95.4%. In 3 of the 4 sites, there was a statistically significant association with age, such that lower percentages were apparent in older children, i.e. in those with the longest duration since initial vaccination.

2. Among all vaccinated and unvaccinated children from 4 sites ( $n = 2040$ ), the overall HBsAg positivity ranged from 0.5% to 4.3%. Among two sites with adequate numbers to directly compare vaccinated and unvaccinated with a clinical endpoint, the efficacy of vaccination was 65-72%. This indicated that the vaccine was having a significant effect in reducing disease incidence, but that also many children were getting disease despite vaccination. A third site revealed minimal disease prevalence in either vaccinated or unvaccinated subjects. The pooled data of the three sites with data on both vaccinated and unvaccinated children revealed an efficacy of 65%, significantly lower than the expected 95% level ( $p < 0.01$ , chi square). A fourth site has not to date enrolled unvaccinated control children, but the disease burden among those with vaccination was very high (4.2%). Moreover, the prevalence of HBsAg+ mothers at this site was not significantly different from any other site, implying that the population prevalence was not substantially higher than elsewhere in the country. Hence, it is likely that vaccine efficacy for children at this 4<sup>th</sup> site will ultimately prove to be significantly lower than other sites.

3. Particularly notable among site-to-site variability in child serology was the significantly lower prevalence of HBsAg positivity observed at the third site, Addis Ababa, compared with the other sites.

4. In parallel to this, participating mothers ( $n = 1789$ ) of the children were evaluated for HBsAg, anti-HBc positivity and anti-Hepatitis C Virus (HCV) positivity. Percentages of HBsAg positivity varied from 3.7 to 5.9%, anti-HBc positivity from 30.5-36.6%, and anti-HCV positivity from 1.1-2.5%. These values are consistent with a large number of previous studies in Ethiopia (9). The constancy of the mothers' serological results from site to site and with previous studies represents an important internal control in the project, because it contrasts dramatically with the greater variability seen from site to site in the pediatric cohorts.

This gives us confidence that there were likely no major technical errors during the pediatric evaluation. In addition, all sites observed that older women, while having high frequency of individuals positive for anti-HBc (indicating HBV infection history), appeared to have lower frequencies of HBsAg positivity or chronic disease. This finding may have significant relevance to the prediction of rates of long-term complications in the country (see below).

It must be stressed that we still consider these results preliminary. The study is on-going, more samples will be collected, additional quality controls will be run, and there have been methodological limitations as described by the authors (1-8). That said, there are several points to be discussed from the data thus far obtained.

#### ***Impact of reduced seroprevalence of anti-HBsAg***

First, the overall fraction of individuals with "protective" levels of antibodies (60%) at first glance may appear to be relatively low. However, comparable percentages have in fact been found in other African studies with subjects in about this same age range after vaccination, and also in reports from Western and Asian cohorts (15-22). While the levels of anti-HBs early after vaccination are very good predictors of efficiency of the vaccine in protecting against disease (24,25), waning antibody levels many years after vaccination are more difficult to interpret (20). Two lines of evidence suggest that clinical efficacy may persist even with undetectable levels of anti-HBsAg and have been used as arguments against need for further vaccine boosters (15,20). First, populations with undetectable antibody 10-15 years after vaccination still exhibit very low levels of disease consistent with existing clinical protection (15,17-19). However, this argument assumes that exposure risks are identical 10-15 years after vaccination compared with early after vaccination, an assumption which may not be valid if national hepatitis prevalence falls after vaccine uptake. Secondly, booster shots in such seronegative individuals yield strong rapid antibody responses consistent with pre-existing persistent HBV-specific memory rather than a de-novo primary response which typically exhibits weak responses initially (15). The presence of memory implies that protective immune responses (including high levels of antibodies) would be rapidly induced upon infectious HBV exposure and would be capable of clearing or attenuating the infection. However, while most studies have shown good memory responses among vaccinated seronegative individuals, some have shown evidence of waning memory after longer time periods (17).

Furthermore, these assays do not test all relevant components of the immune response. There is strong evidence in chimpanzee models (26)—arguably the best animal model of disease—and circumstantial evidence in humans (27), that protective immunity requires a cell-mediated CD8 T cell response. Memory CD8 responses may wane independently of memory for antibody responses and would not have been tested in the aforementioned boosting assays. To our knowledge, no studies have evaluated the impact of a booster vaccine in remotely vaccinated subjects in a prospective study with disease as the primary outcome in a setting where infection risk remains high. Such a study may be worth considering in the Ethiopian context.

#### *Apparent reduced efficacy of vaccine*

Secondly, and more concerning, was the finding that the apparent clinical efficacy of the vaccine, that is the efficacy in reducing the prevalence of HBsAg positivity, 65% or less in this study, is clearly suboptimal. It seems unlikely that any country using vaccination as a strategy to reduce disease will attain the WHO goal of 90% reduction of new cases by 2030 with such suboptimal efficacy (28). Moreover, according to mathematical models (29), a change from 95% to 65% in vaccine efficacy would reduce future preventable mortality not by 80% (as predicted with vaccine efficacy of 95%) but rather about 50% (29). Assuming the aforementioned time-dependent risks of hepatic failure and hepatocellular carcinoma, complications which are nearly invariably fatal, this loss of vaccine efficacy in a country like Ethiopia of 100 million people would result in an additional 5,000 deaths per year. Confirmation of this finding as well as research to define the impact of this disease from an economic, social and health care perspective are warranted. From an etiological perspective, suboptimal efficacy could be related to any one or a combination of the following factors: suboptimal vaccine upon import into Ethiopia, suboptimal storage and/or transport of the vaccine, suboptimal vaccination procedures or administration, or suboptimal host responses to the vaccine. Discriminating between these possibilities in the Ethiopian context will require further research, but it is relevant at present to further discuss in detail one of these, *suboptimal vaccination procedures*.

To gain insight into the impact of the vaccine in the Africa context, a recent review summarized data on HBsAg prevalence from multiple studies focused on specific regions or health care centers within African countries (30). Because these studies were not national surveys, they are not necessarily representative of any given country as a whole; in addition, few of the studies compared similarly aged vaccinated and unvaccinated children at the same time as we have done here.

Nonetheless the summarized data does suggest substantial reductions in HBsAg positivity among children in the continent as a whole. However there have been many studies in locations exhibiting child seroprevalences > 2.0% after vaccination, similar to what we are describing here in Ethiopia, implying suboptimal vaccine impact. Discussions of these results in recent reviews to identify means of improvement have primarily focused on the common practice of immunizing neonates, not at birth—a practice which has been shown to have the highest efficacy—but several weeks after birth (30,31). Immunization at birth is relevant for those infants who are already infected by HBV infected mothers, i.e. by vertical transmission. A large number of clinical trials have evaluated multiple HBV vaccination protocols. Fewer have evaluated efficacy when administered at later time points, but those trials suggest an efficacy of 50-75% among those with prior vertically transmitted disease, rather than 95% when given at birth (32). Ethiopia is one of several countries which immunizes at 6, 10, and 14 weeks after birth. For a country with traditionally little antenatal care, particularly in rural settings, this regiment is arguably not unreasonable.

Could the reduced efficacy of delayed vaccination explain the results we have obtained in our current study to date? Probably not is the short answer, but it is instructive going into this in more detail. Most of the available data in Africa relevant to vertical transmission were prospective studies done in the pre-vaccination era (33-35), including one done in Ethiopia by the late Professor Edemariam Tsega who, it must be remarked, pioneered the knowledge base on hepatitis in this country (36). The typical study design involved evaluation of newborns of HBsAg+ and HBsAg- mothers prospectively over several years. Vertical transmission cases were invariably identified within the first year of life (and only among hepatitis positive mothers, and typically among those expressing the serologic marker HBeAg which is associated with high viral load). However, in subsequent years, higher numbers of infants became HBsAg+, indicating that horizontal transmission is the predominant mechanism among young children in Africa (33-36). Interestingly, though vertical transmission is typically stated as the predominant mode of transmission in Asian countries such as China, in fact this generalization is misleading and not supported by the early literature in the pre-vaccine era. Rates of vertical transmission in China are indeed high; without intervention, the majority of children from chronically infected mothers become infected.

However, horizontal transmission has accounted for the majority (50-80%) of cases among children (37,38). Similarly, early studies in Alaska, where chronic hepatitis among children was highly prevalent in the pre-vaccine era, pointed to horizontal transmission as the principal mechanism. Thus, horizontal transmission may well be the prominent mechanism globally, not just in Africa.

More recent studies have implied greater contribution of vertical transmission, but these studies are often over-represented by those focusing on HIV infected women (39) and would not be surprising that rates of vertical transmission are indeed higher in this group, owing to reduced maternal immune function and higher hepatitis B viral load. Most pregnant HBsAg+ women in Africa, however, are HIV negative, so studies of HIV+ women are not representative of most women. Furthermore, an arguably simpler strategy for HIV+ pregnant women would be appropriate screening for HBsAg and switching positive mothers to an ART regimen including tenofovir which has dual activity against HIV and HBV (12), thus providing better treatment and prevention for both mother and child, respectively. In addition to studies on HIV+ women, many recent studies have also argued for high maternal to child transmission rates based on assays of HBsAg levels in cord blood or the blood of infants in the first months of life (30). However, it has been clearly demonstrated that HBsAg from maternal sources readily crosses the placenta and only slowly disappears during the first few months among infants who subsequently show no evidence of disease either by HBsAg or sensitive HBV DNA testing (40,41), indicating that estimates of vertical transmission by such HBsAg testing among newborns are unreliable.

Collectively, these points reinforce that horizontal, not vertical transmission is likely the main mechanism explaining high prevalence of childhood chronic hepatitis. To argue otherwise would require proposal of a quite novel mechanism, and one which would be difficult to test in humans at least. Perinatally infected babies would somehow have to be able to initially confine HBV infection to liver or other sites, perhaps due to a very low inoculum, such that infection was uncommonly apparent in blood, but only much later emerged, presumably after years of slow net HBV replication. This is theoretically plausible but implies an element of immune control likely beyond that known in neonates who generally have immature immune systems, and at this point seems unlikely.

Despite the preponderance of evidence for primarily horizontal transmission, it is clear, however, that vertical transmission does exist.

Assuming *delayed* birth vaccination would proceed *indefinitely* and with the *best possible efficacy* (ie with 50-75% protection against vertical and 95% against horizontal transmission), then eventually future residual cases *would* likely result from suboptimally protected neonates from vertical transmission. Owing to the demonstrably greater efficacy of birth vaccination, it stands to reason that wherever feasible within national resources, this should be the preferred mode of immunization at the global level to obtain the maximum reduction of infection and disease.

However, are all delayed birth vaccinations currently exhibiting the *best possible efficacy*? How does this relate to our study findings of reduced vaccine efficacy in Ethiopia? Assuming predicted efficacy among vertically transmitted HBsAg+ newborns with delayed vaccination in previous trials (50-75%), it seems unlikely that the reduced vaccine efficacy we have seen to date in Ethiopia (65% or less) is solely due to post-birth vaccine delay, because that would require nearly all cases to have been vertically acquired, which itself is unlikely. It seems something else is going on. This argument may or may not apply to other African countries.

To obtain more evidence relevant to this issue, we are performing molecular sequencing studies on strains from paired HBsAg+ mother and child samples. Paired strains which are molecularly identical most likely would have been transmitted from mother to child. Molecularly disparate paired strains imply horizontal transmission from a non-maternal source. Interestingly, one recent study used a similar molecular approach evaluating strains circulating within and between families and found no evidence of vertical transmission in young children in a remote village in Gabon (42). It remains to be seen what this approach will reveal in Ethiopia, but if evidence for significant maternal transmission is found, this approach may further be used to determine whether vaccine efficacy is reduced among such children compared with children infected by non-maternal sources.

If insufficient evidence is obtainable to implicate primarily the post-birth vaccine schedule as the main factor contributing to suboptimal vaccine efficacy in Ethiopia (which appears to be the case so far), this implies the involvement of other factors, such as reduced vaccine quality upon import, improper vaccine transport and storage, inadequate vaccine administration (beyond post-birth regimen issues), and/or suboptimal host immune responses to the vaccine. Research into these other possibilities should be considered.

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#### ***Regional differences in HBsAg+ prevalence among children***

Thirdly, a remarkable finding in our current study was that there were no detectable HBsAg positive children from Addis Ababa among non-vaccinated children, although recruitment numbers were lower than in other sites. Notably, we observed the same finding in a pilot study in Addis Ababa at the ALERT hospital done 2 years previously (Yonas Bekele, unpublished observations), giving us confidence that this finding may well be true. The pooled data from these two recent studies in Addis Ababa differs significantly from the combined data from the other sites ( $p < 0.01$ , chi square). Moreover, the low level of pediatric chronic HBV disease in Addis Ababa we observed in recent years contrasts markedly with the percentages observed in 1995 in Addis Ababa (~4%), a value closer to the percentages seen in among unvaccinated children in multiple peripheral sites in Ethiopia in the current study. Although definitive conclusions based on different sites and different years is not possible, at face value this suggests that as yet unidentified factors associated with Addis Ababa, but not other regions in the country, have changed over the past twenty years, and these factors contribute to significantly lower rates of hepatitis independently of the vaccine, an extraordinary possibility.

Of relevance to the heterogeneity of HBsAg+ prevalence in different cities in Ethiopia are several studies regarding hepatitis cases from within or following emigration from China, where as stated previously, horizontal transmission has played a prominent role. Differences between different cities, and between urban and rural locations (37) have been apparent. For example, in the pre-vaccine era, prevalence of chronic disease among children was dramatically lower in Beijing than in the southern province of Guangdong, with virtually all the differences accountable to children older than 1 year of age (37), i.e. those who would have been horizontally infected. Most of the factors that impacted horizontal transmission were related to close immediate family contact with known HBsAg+ carriers. However, in interventional studies use of sterilized syringes dramatically reduced prevalence among children (36), suggesting the involvement of multiple factors. Apart from these studies, a unique study in the pre-vaccine era was reported many years ago of Chinese immigrants to South Africa (43). Nearly all were from the Guangdong province, and most settled in all-white neighborhoods in South Africa with high standards of living and access to high levels of education and health care, and in which HBsAg prevalence was previously negligible. A cross-sectional analysis of multiple age groups indicated that the immigrants born in China had a very high prevalence of HBsAg positivity, similar to that of Guangdong province, but their children, born in South Africa had dramatically lower prevalence, and much lower than similarly aged individuals in Guangdong province at the time. Collectively, these studies underscore the impact that regional differences within countries can have on HBsAg prevalence, particularly due to horizontal transmission. It may be of great public health importance to further confirm the finding of regional differences in Ethiopia, and if true, begin to probe the factors associated with such differences. Identifying and controlling such factors would greatly enhance the ability of the country to meet WHO goals of 90% reduction of new hepatitis B cases by 2030.

#### ***Low HBsAg expression among older hepatitis infected women***

Fourthly, the finding that a significantly lower percentage of older mothers than younger mothers were HBsAg positive, even though they had at least as high an infection prevalence (as defined by anti-HBc positivity) as younger mothers, is also of potential importance. Most individuals who become HBsAg positive remain HBsAg positive, though many will shift from HBeAg positive to negative in association with a large reduction in viral load as the immune system gains control over the chronic infection (36).

Among HBsAg+ individuals, a small percentage, ~1-2%/year, “resolve” the infection and become negative for HBsAg, maintaining anti-HBc levels (45-47). The percentages of HBsAg positive individuals among older anti-HBc positive women in Ethiopia were much lower than that expected based on the aforementioned studies. It is possible that, compared with other settings, in Ethiopia a much higher percentage of HBsAg+ individuals resolve the infection/year. This would account for the reduced HBsAg+ percentage in older women, assuming they had the infection longer than younger women. Importantly, studies suggest those with chronic HBV who resolve the infection have lower risks of long-term disease complications (48).

This possibility in Ethiopia would ideally be defined in longitudinal studies, but there is a paucity of data in Ethiopia or Africa on the natural evolution of hepatitis disease. Virtually all studies have been done in Western and SE Asian contexts.

Studies in non-African settings have shown that disease complications are significantly impacted by the strain of hepatitis virus (49-50). Moreover, it is well established that strain distribution differs substantially at the global level (though all strains share the “a” antigen determinant present in the vaccine) (51). The most striking findings have been observed in China comparing strains B and C (49). Strain C is associated with higher viral load and HBeAg expression, higher vertical transmission as well as higher risks for long term complications such as cirrhosis and hepatocellular carcinoma. Studies on clinical associations with strains circulating in Africa, such as A and D—those most likely circulating in Ethiopia (52)—have not been done in Africa but rather in the US where strains A, B, C, and D all circulate (50). These results were less straightforward than the China studies but mostly suggest that strains A and D are more similar to strain B than to the more virulent strain C common in China and SE Asia (47).

These findings are consistent with lower frequencies of HBeAg expression in HBsAg carriers observed in Africa in general (39) and Ethiopia in particular (9), as well as lower rates of vertical transmission, compared with China and SE Asia. The aforementioned hypothesis that in Ethiopia resolution and clearance of HBsAg proceeds more efficiently than in other settings would also be consistent with reduced risk for long term complications such as cirrhosis and hepatocellular carcinoma among HBsAg+ individuals. This view, however, should by no means trivialize the overall burden of cirrhosis and hepatocellular carcinoma in Ethiopia which is none-the-less very large presumably because of the high prevalence of HBsAg+ individuals (9).

Rather, it underscores the need to better define the disease evolution and complications, particularly utilizing molecular and longitudinal approaches in combination with protocols defining optimal treatment initiation and maintenance. This in turn can assist in prioritizing allocation of national resources.

In summary, we are well into a large multi-site study in Ethiopia to define the hepatitis B burden, particularly among children in the country, and the effectiveness of the HBV vaccination program initiated several years ago. While recruitment and data analysis is not yet finalized, several key findings appear to have emerged thus far: 1) HBV vaccination has apparently reduced childhood disease, but vaccine efficacy appears to be significantly below targets assumed in global models of hepatitis B virus control, and this reduced efficacy cannot be explained by the delayed birth vaccination protocol in use; 2) Marked regional differences in both disease and vaccine efficacy are apparent; in particular, for unknown reasons, the prevalence of childhood chronic hepatitis in *unvaccinated* children in Addis Ababa is apparently substantially lower than that defined in Addis Ababa 20 years ago, as well as significantly lower than that in other regions in the country. Future confirmation of these findings and research into their underlying mechanisms, longitudinal studies into the natural evolution of disease in Ethiopia, and impact of booster vaccines in disease protection should be considered.

## REFERENCES

1. Geta M, Ayalew G, Yizengaw E, Mihret A, Aseffa A, Howe R et al. Seroprevalence of hepatitis B virus infection and associated factors among mothers in Gondar, northwest Ethiopia: A population based study. *Ethiop Med J*, 2019, Supp. 2.
2. Taye BD, Kassa T, Teshager L, Kedir R, Yeshanew AG, Aseffa A et al. Prevalence and associated risk factors of hepatitis B and C virus infections among mothers in Jimma, southwest Ethiopia: A community-based study. *Ethiop Med J*, 2019, Supp. 2.
3. Abebe F, Seyoum B, Teklemariam Z, Oljira L, Tarekegne A, Bekele F et al. Seroprevalence of hepatitis B virus surface antigen, anti-hepatitis C virus antibody and their associated factors among mothers living in Harar, eastern Ethiopia. *Ethiop Med J*, 2019, Supp. 2.
4. Biazin H, Teshome S, Ayenew Z, Abebe T, Mihret A, Aseffa A et al. Determining seroprevalence of hepatitis B and C virus infections and associated risk factors among apparently healthy mothers in Addis Ababa, Ethiopia. *Ethiop Med J*, 2019, Supp. 2.
5. Getnet Ayalew A, Abate Assefa A, Anteneh Amsalu A, Mekuanint Geta M, Adane Mihret A, Abraham Aseffa A, et al. Immune protection of hepatitis B vaccine among children living in Gondar, northwest Ethiopia. *Ethiop Med J*, 2019, Supp. 2.
6. Kedir R, Taye BD, Kassa T, Teshager L, Aseffa A, Howe R et al. Seroprevalence of hepatitis B virus infection and seroprotection of hepatitis B vaccine among children in Jimma town, southwest Ethiopia. *Ethiop Med J*, 2019, Supp. 2.
7. Usman S, Seyoum B, Mengiste B, Tarekegne A, Bekele F, Mihret A et al. Serum levels of anti-hepatitis B surface antigen and HBV associated factors among vaccinated and unvaccinated children in Harar, eastern Ethiopia: A community-based study. *Ethiop Med J*, 2019, Supp. 2.
8. Teshome S, Biazin H, Tarekegne A, Abebe T, Bekele F, Mihret A et al. Antibody response against hepatitis B virus after vaccination and seroprevalence of HBV in children in Addis Ababa, Ethiopia. *Ethiop Med J*, 2019, Supp. 2.
9. Belyhun Y, Maier M, Mulu A, Diro E, Liebert UG. Hepatitis viruses in Ethiopia: a systematic review and meta-analysis. *BMC Infectious Diseases*. 2016;16:761.
10. Fattovich G, Bortolotti F, Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. *J Hepatol*. 2008;48(2):335.
11. Guidelines for the care and treatment of persons diagnosed with chronic hepatitis C virus infection. Geneva: World Health Organization. 2018.
12. Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection. Geneva: World Health Organization. 2015.
13. Abdelwahab SK, Ahmed Said ZN. Status of hepatitis C virus vaccination: Recent update. *World J Gastroenterol*. 2016 January 14; 22(2): 862-873.
14. Spearman CW, Afihene M, Ally R, Apica B, Awuku Y, Cunha L et al. Hepatitis B in sub-Saharan Africa strategies to achieve the 2030 elimination targets. *Lancet Gastroenterol Hepatol*. 2017; 2: 900-0.
15. Fitzsimons D, François G, Hall A, McMahon B, Meheus A, Zanetti A et al. Long-term efficacy of hepatitis B vaccine, booster policy, and impact of hepatitis B virus mutants. *Vaccine*. 2005 Jul 14;23(32):4158-66.
16. Liao SS, Li RC, Li H, Yang JY, Zeng XJ, Gong J, Wang SS, Li YP, Zhang KL. Long-term efficacy of plasma-derived hepatitis B vaccine: a 15-year follow-up study among Chinese children. *Vaccine*. 1999 Jun 4;17(20-21):2661-6.
17. Samandari T, Fiore AE, Negus S, Williams JL, Kuhnert W, McMahon BJ, Bell BP. Differences in Response to a Hepatitis B Vaccine Booster Dose Among Alaskan Children and Adolescents Vaccinated During Infancy. *Pediatrics*. 2007 Aug;120(2):e373.
18. Rey-Cuille M-A, Seck A, Njouom R, Chartier L, Sow HD, et al. Low Immune Response to Hepatitis B Vaccine among Children in Dakar, Senegal. *PLoS ONE*. 2002; 7(5): e38153.
19. van der Sande MAB, Waight P, Mendy M, Rayco-Solon P, Hutt P, Fulford T et al. Long-Term Protection against Carriage of Hepatitis B Virus after Infant Vaccination. *J Infect Dis*. 2006 June; 139(11): 1528-1535.
20. Van Damme P. Long-term Protection After Hepatitis B Vaccine. *J Infect Dis*. 2016 July; 214(1):1-3.
21. Tsebe KV, Burnett RJ, Hlungwani NP, Sibara MM, Venter PA, Mphahlele MJ. The first five years of universal hepatitis B vaccination in South Africa: evidence for elimination of HBsAg carriage in under 5-year-olds. *Vaccine*. 2001 Jul 16;19(28-29):3919-26.
22. Odusanya OO, Alufohai E, Meurice FP, Ahonkhai VI. Five-year post vaccination efficacy of hepatitis B vaccine in rural Nigeria. *Hum Vaccin*. 2011 Jun;7(6):625-9.

23. Abebe A, Nokes DJ, Enquesslassie, Messele T, Cutts FT. Seroepidemiology of hepatitis B virus in Addis Ababa, Ethiopia: transmission patterns and vaccine control. *Epidemiol. Infect.* 2003;131:757–770.
24. Jack AD, Hall AJ, Maine N, Mendy M, and H. C. Whittle. What Level of Hepatitis B Antibody Is Protective? *J Infect Dis.* 1999;179:489–92.
25. Centers for Disease Control. Recommendations of the Immunisation Practices Advisory Committee. Recommendations for protection against viral hepatitis. *MMWR.* 1985; 34: 329–35.
26. Timme F, Widland S, Steiger C, Ghayeb J, Reimann KA, Purcell RH, Chisari FV. CD8 T cells mediate viral clearance and disease pathogenesis during acute hepatitis B infection. *J Viology.* 2003; 77(1):68-76.
27. Maini MK, Boni, C, Lee CK, Larrubia JR, Reignat S, Ogg GS et al. The role of virus-specific CD8+ cells in liver damage and viral control during persistent hepatitis B Virus infection. *JEM.* 2000;191(8):1269-1280.
28. Global health sector strategy on viral hepatitis 2016-2021 towards ending viral hepatitis. Geneva: World Health Organization. 2016.
29. Goldstein ST, Zhou FJ, Hadler SC, et al. A mathematical model to estimate global hepatitis B disease burden and vaccination impact. *Int J Epidemiol.* 2005; 34:1329–39.
30. Breakwell L, Tevi-Benissan C, Childs L, Mihigo R, Tohme R. The status of hepatitis B control in the African region. *Pan Afr Med J.* 2017 Jun 22;27(Suppl 3):17.
31. Spearman CW, Afihene M, Ally R, Apica B, Awuku Y, Cunha L et al. Hepatitis B in sub-Saharan Africa: strategies to achieve the 2030 elimination targets. *Gastroenterology and Hepatology Association of sub-Saharan Africa (GHASSA). Lancet Gastroenterol Hepatol.* 2017 Dec;2(12):900-909.
32. Nelson NP, Easterbrook MJ, McMahon BJ. Epidemiology of Hepatitis B Virus Infection and Impact of Vaccination on Disease. *Clin Liver Dis.* 2016 November; 20(4): 607–628.
33. Marinier E, Barrois V, Larouze B, et al. Lack of perinatal transmission of hepatitis B virus infection in Senegal, West Africa. *J Pediatr* 1985; 106: 843–9.
34. Greenfield C, Osidiana V, Karayiannis P, et al. Perinatal transmission of hepatitis B virus in Kenya: its relation to the presence of serum HBV-DNA and anti-HBe in the mother. *J Med Virol.* 1986;19:135–42.
35. Menendez C, Sanchez-Tapias JM, Kahigwa E, et al. Prevalence and mother-to-infant transmission of hepatitis B, C, and E viruses in Southern Tanzania. *J Med Virol.* 1999; 58: 215–20.
36. Tsega E, Tsega M, Mengesha B, Nordenfelt E, Hansson BG, Lindberg J. Transmission of hepatitis B virus infection in Ethiopia with emphasis on the importance of vertical transmission. *Int J Epidemiol.* 1988;17: 874–9.
37. Yao JL. Perinatal transmission of hepatitis B virus infection and vaccination in China. *Gut.* 1996; 38 (suppl 2): S37-S38.
38. Yao GB. Importance of perinatal versus horizontal transmission of hepatitis B virus infection in China. *Gut.* 1996; 38 (suppl 2): S39-S42.
39. Keane E, Funk AL, Shimakawa Y. Systematic review with meta-analysis: the risk of mother-to-child transmission of hepatitis B virus infection in sub-Saharan Africa. *Aliment Pharmacol Ther.* 2016; 44: 1005–1017.
40. Papaevangelou V, Paraskevis D, Anastassiadou V, Stratiki E, Machaira M, Pitsouli et al. HBV viremia in newborns of HBsAg(+) predominantly Caucasian HBeAg(-) mothers. *Journal of Clinical Virology.* 2011;50: 249–252.
41. Papaevangelou, G., Kremastinou, T., Prevedourakis, C., and Kaskarelis, D. Hepatitis B antigen and antibody in maternal blood, cord blood, and amniotic fluid. *Archives of Disease in Childhood.* 1974. 49:936.
42. Sandrine François-Souquière AF, Makuwa M, Bisvigou U, Kazanji M. Epidemiological and molecular features of hepatitis B and hepatitis delta virus transmission in a remote rural community in central Africa. *Infection, Genetics and Evolution.* 2016; 39:12–21.
43. Song E, Kew MC, Hwang L-Y, and Beasley RP. Epidemiology of hepatitis B virus infection in South African Chinese. *Am J Epidemiol.* 1988; 128:828-38.
44. Croagh CMN, Lubel JS. Natural history of chronic hepatitis B: Phases in a complex relationship. *World J Gastroenterol.* 2014 August 14; 20(30): 10395-10404.
45. Alward WL, McMahon BJ, Hall DB, Heyward WL, Francis DP, Bender TR. The long-term serological course of asymptomatic hepatitis B virus carriers and the development of primary hepatocellular carcinoma. *J Infect Dis.* 1985;151(4):604.
46. Liaw YF, Sheen IS, Chen TJ, Chu CM, Pao CC. Incidence, determinants and significance of delayed clearance of serum HBsAg in chronic hepatitis B virus infection: a prospective study. *Hepatology.* 1991;13(4):627.
47. Liu J, Yang HI, Lee MH, Lu SN, Jen CL, Wang LY et al. Incidence and determinants of spontaneous hepatitis B surface antigen seroclearance: a community-based follow-up study. *Gastroenterology.* 2010;139(2):474.

47. Simonetti J, Bulkow L, McMahon BJ, Homan C, Snowball M, Negus S et al. Clearance of Hepatitis B Surface Antigen and Risk of Hepatocellular Carcinoma in a Cohort of Chronically Infected with Hepatitis B Virus. *Hepatology*. 2010;51:1531-1537.
49. Yuen MF, Sablon E, Yuan HJ, et al. Significance of hepatitis B genotype in acute exacerbation, HBeAg seroconversion, cirrhosis-related complications, and hepatocellular carcinoma. *Hepatology*. 2003; 37:562.
50. Chu CJ, Keeffe EB, Han SH, et al. Hepatitis B virus genotypes in the United States: results of a nationwide study. *Gastroenterology*. 2003; 125:444.
51. Norder H1, Hammas B, Lee SD, Bile K, Couroucé AM, Mushahwar IK, Magnus LO. Genetic relatedness of hepatitis B viral strains of diverse geographical origin and natural variations in the primary structure of the surface antigen. *J Gen Virol*. 1993 Jul;74 (Pt 7):1341-8.
52. Stoyan Velkov S, Ott JJ, Protzer U, Michler T. The Global Hepatitis B Virus Genotype Distribution Approximated from Available Genotyping Data. 2018. *Genes*. 9, 495.